Supplementary data

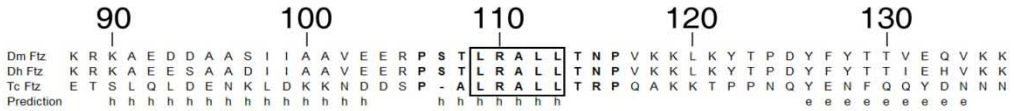
Figure S1. Short FTZ fragments containing the LXXLL motif are sufficient for binding to the FTZ-F1 LBD. (A) A sequence alignment of FTZ proteins from *D. melanogaster* (Dm FTZ), *D. hydei* (Dh FTZ), and *T. castaneum* (Tc FTZ) containing the LXXLL motif (boxed) is shown. Numbering is based on the *D. melanogaster* FTZ. The secondary structure prediction is shown beneath the alignment, where 'h' indicates helix and 'e' indicates β-strand. (B) A schematic of FTZ fragments used in the far-western experiment. The black box indicates the LXXLL motif and the red box indicates the homeodomain. (C) A far-western of bacterially expressed GST and FTZ fragments probed with radiolabeled FTZ-F1 LBD. Lanes 1–5 correspond to the far-western and lanes 6–10 correspond to the Coomassie-stained gel showing the amounts of protein loaded. Lanes 1, 6: GST; lanes 2, 7: GST-FTZ 88–119; lanes 3, 8: GST-FTZ 88–136; lanes 4, 9: FTZ 1–170; lanes 5, 10: FTZ.

Figure S2. Quantification of FTZ^{PEP} binding to FTZ-F1 LBD. (A) SPR sensorgrams of FTZ^{PEP} binding to immobilized FTZ-F1 LBD. FTZ^{PEP} concentrations injected over the sensor chip were 12.5 μM (trace a), 25 μM (trace b), 50 μM (trace c), 100 μM (trace d), and 200 μM (trace e). (B) Raw data from isothermal titration calorimetry (upper panel) and enthalpy changes per mole plotted as a function of the molar ratio of FTZ^{PEP} to FTZ-F1 LBD (lower panel). The solid line represents the best-fit curve. FTZ^{PEP} (0.83 mM) was injected into a 1.4-mL cell containing 0.03 mM FTZ-F1 LBD at 25°C. The values of the best-fitting parameters are 0.944 for N, 6.60×10^5 M^{-1} for K (1.52×10^{-6} M for K_d) and -7.568 kcal/mol for ΔH.

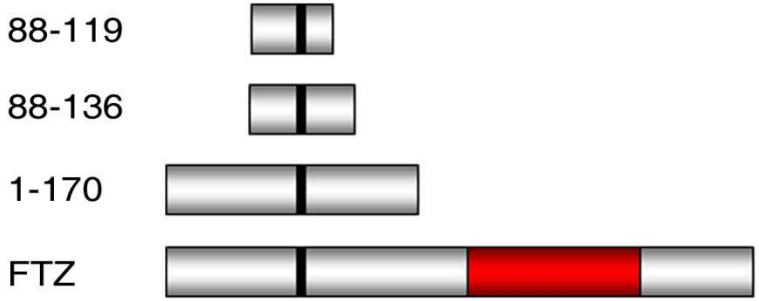
Figure S3. Circular dichroism spectra of FTZ-F1 LBD and mutant proteins. Far-UV CD spectra of FTZ-F1 LBD wild type and mutants were measured in 20 mM Tris-HCl pH7.5 at approximately 0.1-0.5 mg/ml. Average values are expressed as millidegree at various wavelengths, high values were found at high protein concentrations. CD spectra suggest that all mutants have α-helical structures, which is consistent with X-ray crystal structure of the wild type FTZ-F1 LBD.

Figure S4. Amino acid sequence alignment and secondary structures of the FTZ-F1, LRH-1, and SF-1 LBD. Asterisk (*) indicates residues between β1 and β2 which are not conserved in the LRH-1, and SF-1 LBDs. Secondary structures and primary sequences are well conserved except residues in the loop between β sheet.

A


90
100
110
120
130
Dm Ftz K R K A E D D A A S I I A A V E E R P S T L R A L L T N P V K K L K Y T P D Y F Y T T V E Q V K K
Dh Ftz K R K A E E S A A D I I A A V E E R P S T L R A L L T N P V K K L K Y T P D Y F Y T T I E H V K K
Tc Ftz E T S L Q L D E N K L D K K N D D S P - A L R A L L T R P Q A K K T P P N Q Y E N F Q Q Y D N N N
Prediction h h h h h h h h h h h h h h h h h h h h e e e e e e e e e

B


88-119
88-136
1-170
FTZ

C

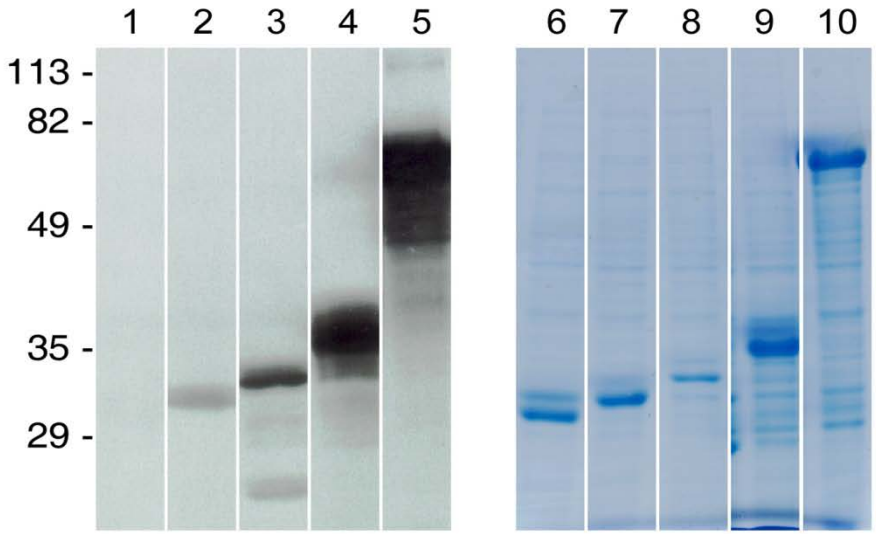
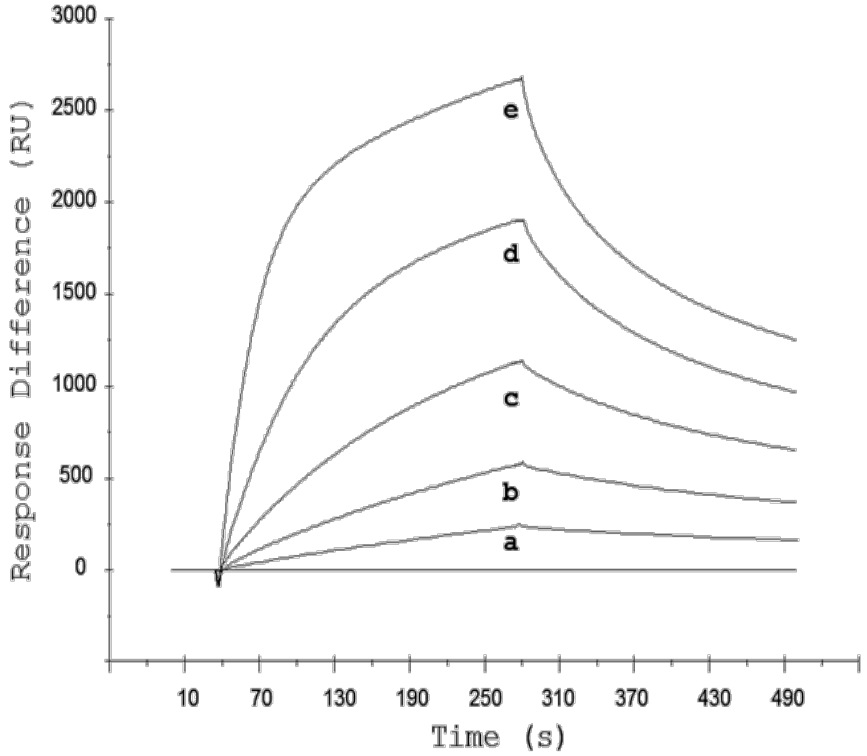

1 2 3 4 5
6 7 8 9 10
113 -
82 -
49 -
35 -
29 -

Fig. S1

A


Response Difference (RU)
3000
2500
2000
1500
1000
500
0
10
70
130
190
250
310
370
430
490
Time (s)
e
d
c
b
a

B

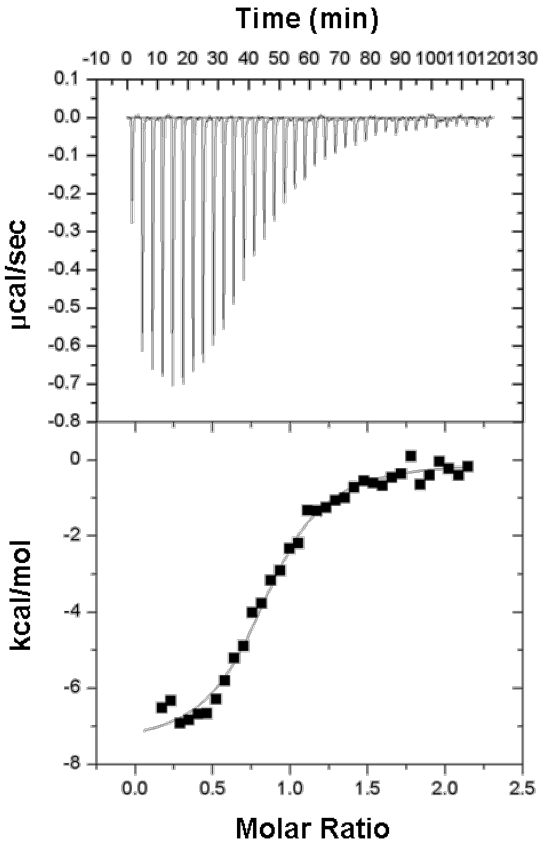
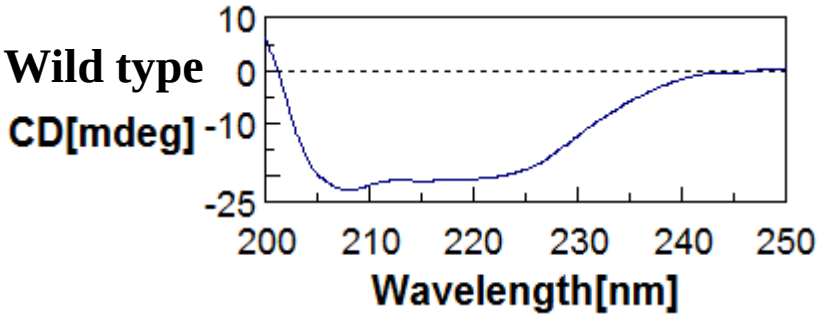

Time (min)
-10 0 10 20 30 40 50 60 70 80 90 100 110 120 130
µcal/sec
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
kcal/mol
0
-2
-4
-6
-8
0.0
0.5
1.0
1.5
2.0
2.5
Molar Ratio

Fig. S2


Wild type
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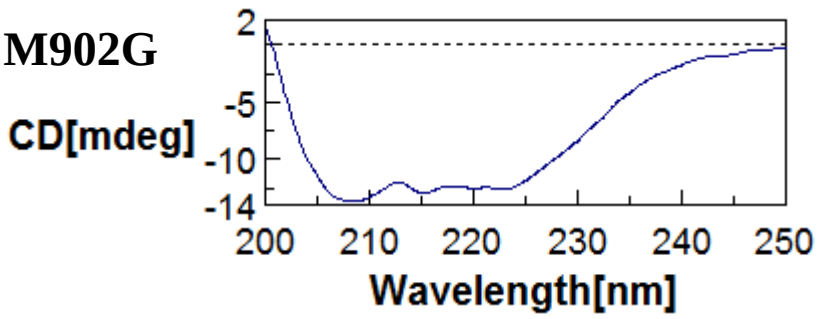
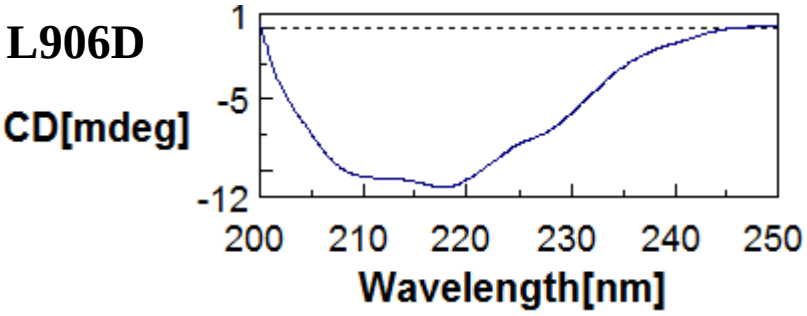
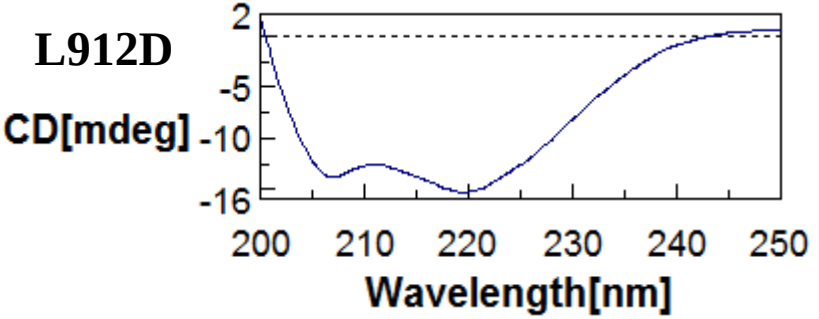
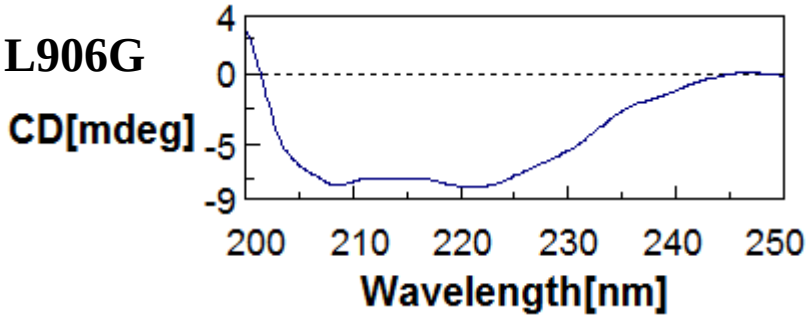
10
0
-10
-25
200 210 220 230 240 250
Wavelength[nm]


M902G
CD[mdeg]
2
-5
-10
-14
200 210 220 230 240 250
Wavelength[nm]


L906D
CD[mdeg]
1
-5
-12
200 210 220 230 240 250
Wavelength[nm]


L912D
CD[mdeg]
2
-5
-10
-16
200 210 220 230 240 250
Wavelength[nm]


L906G
CD[mdeg]
4
0
-5
-9
200 210 220 230 240 250
Wavelength[nm]

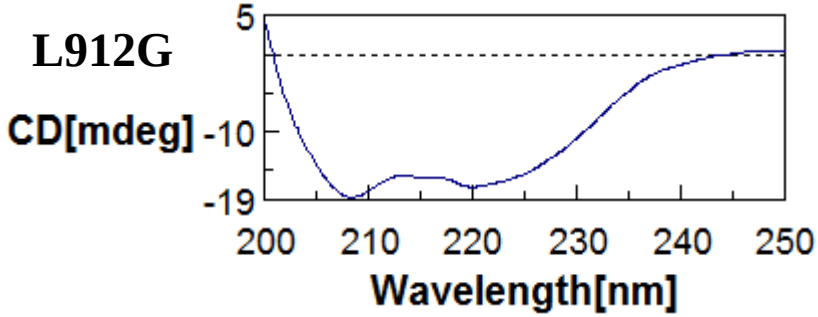
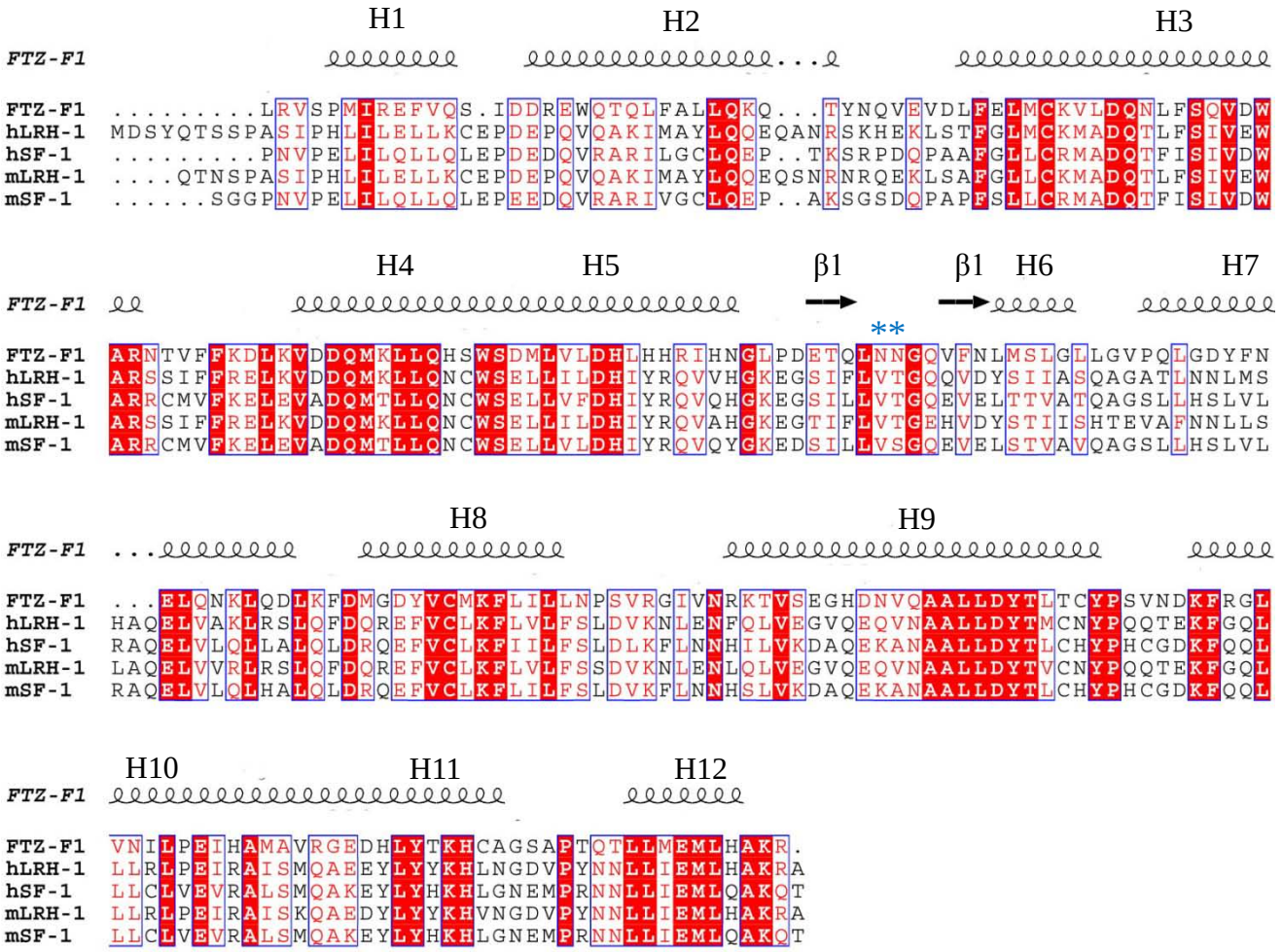

L912G
CD[mdeg]
5
-10
-19
200 210 220 230 240 250
Wavelength[nm]

Fig. S3


H1
H2
H3
FTZ-F1
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hLRH-1 MDSYQTSSPASIPHLILELLKCEPDEPQVQAKIMAYLQQEQANRSKHEKLSTFGLMCKMADQTLFSIVEW
hSF-1 .....PNVPELILQLLQLEPDEDQVRARILGCLQEP..TKSRPDQPAAFGLLCRMADQTFISIVDW
mLRH-1 ....QTNSPASIPHLILELLKCEPDEPQVQAKIMAYLQQEQSNRNRQEKLSAFGLLCKMADQTLFSIVEW
mSF-1 ......SGGPNVPELILQLLQLEPEEDQVRARIVGCLQEP..AKSGSDQPAPFSLLCRMADQTFISIVDW
H4
H5
β1
β1
H6
H7
FTZ-F1
**
FTZ-F1 ARNTVFFKDLKVDDQMKLLQHSWSDMLVLDHLHHRIHNGLPDETQLNNGQVFNLMSLGLLGVPQLGDYFN
hLRH-1 ARSSIFFRELKVDDQMKLLQNCWSELLILDHIYRQVVHGKEGSIFLVTGQQVDYSIIASQAGATLNNLMS
hSF-1 ARRCMVFKELEVADQMTLLQNCWSELLVFDHIYRQVQHGKEGSILLVTGQEVELTTVATQAGSLLHSLVL
mLRH-1 ARSSIFFRELKVDDQMKLLQNCWSELLILDHIYRQVAHGKEGTIFLVTGEHVDYSTIISHTEVAFNNLLS
mSF-1 ARRCMVFKELEVADQMTLLQNCWSELLVLDHIYRQVQYGKEDSILLVSGQEVELSTVAVQAGSLLHSLVL
H8
H9
FTZ-F1
FTZ-F1 ...ELQNKLQDLKFDMGDYVCMKFLILLNPSVRGIVNRKTVSEGHDNVQAALLDYTLTCYPSVNDKFRGL
hLRH-1 HAQELVAKLRSLQFDQREFVCLKFLVLFSLDVKNLENFQLVEGVQEQVNAALLDYTMCNYPQQTEKFGQL
hSF-1 RAQELVLQLLALQLDRQEFVCLKFIILFSLDLKFLNNHILVKDAQEKANAALLDYTLCHYPHCGDKFQQL
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mSF-1 RAQELVLQLHALQLDRQEFVCLKFLILFSLDVKFLNNHSLVKDAQEKANAALLDYTLCHYPHCGDKFQQL
H10
H11
H12
FTZ-F1
FTZ-F1 VNILPEIHAMAVRGEDHLYTKHCAGSAPTQTLLMEMLHAKR.
hLRH-1 LLRLPEIRAISMQAEEYLYYKHLNGDVPYNNLLIEMLHAKRA
hSF-1 LLCLVEVRALSMQAKEYLYHKHLGNEMPRNNLLIEMLQAKQT
mLRH-1 LLRLPEIRAISKQAEDYLYYKHVNGDVPYNNLLIEMLHAKRA
mSF-1 LLCLVEVRALSMQAKEYLYHKHLGNEMPRNNLLIEMLQAKQT

Fig. S4